

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

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1. AGENCY USE ONLY
(Leave blank)**2. REPORT DATE**
November 2003**3. REPORT TYPE AND DATES COVERED**
Annual Summary (1 Nov 2002 - 31 Oct 2003)**4. TITLE AND SUBTITLE**

Sulfotransferase 1A1 (SULT1A1) Genotype and Phenotype in Relation to Efficacy of Tamoxifen Treatment

5. FUNDING NUMBERS

DAMD17-02-1-0274

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REPORT NUMBER****9. SPONSORING / MONITORING****AGENCY NAME(S) AND ADDRESS(ES)**U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012**10. SPONSORING / MONITORING
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES**

20040421 021

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE**13. ABSTRACT (Maximum 200 Words)**

Genetic polymorphisms in SULT1A1 have been associated with overall survival in breast cancer patients receiving tamoxifen. Studies have suggested that exposure of a breast cancer cell line to 4-hydroxytamoxifen (4-OH TAM) results in the upregulation of SULT1A1 transcript. Up-regulation of transcript may be related to the observed difference in overall survival by SULT1A1 genotype in tamoxifen-treated patients. SULT1A1 is expressed in platelets, and its expression has been shown to be coordinately regulated across tissues. Therefore, we proposed to recruit 150 patients with primary, incident breast cancer and collect a blood sample for platelet isolation and SULT1A1 activity assays both before and after tamoxifen treatment. Forty-nine patients have been recruited to date, and platelets, along with other blood components have been stored for subsequent assays. Additionally, the effect of 4-OH TAM treatment on the breast cancer cell lines MCF-7 and ZR74-1 have been evaluated by RT-PCR. Analysis by this method did not support the idea that SULT1A1 was regulated by tamoxifen. When enzymatic activity assays were performed using cytosol from cells treated with 4-OH TAM, there was no evidence of increased enzymatic activity with this treatment. These investigations will continue with other breast cancer cell lines.

14. SUBJECT TERMS

Sulfotransferase, tamoxifen, breast cancer

15. NUMBER OF PAGES

9

16. PRICE CODE**17. SECURITY CLASSIFICATION
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION
OF ABSTRACT**

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

AD_____

Award Number: DAMD17-02-1-0274

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TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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INTRODUCTION

Antiestrogen therapy is a widely used method of treatment for hormone-responsive breast cancers and tamoxifen is the most common antiestrogen administered in the United States. The pharmacology and metabolism of tamoxifen is complex, and sulfation is a key biotransformation pathway for this antiestrogen. Human sulfotransferase enzymes (SULTs) are widely expressed Phase II detoxification enzymes that catalyze the biotransformation of an extensive number of endogenous compounds and xenobiotics (1). To date, at least ten human cytosolic SULT isoforms have been identified and assigned to two major subfamilies, the phenol SULT1 family and the hydroxysteroid SULT2 family (2). A recent study using gene expression profiling revealed that expression of SULT1A mRNA is up-regulated 10-fold by 4-hydroxytamoxifen (4-OH TAM) in the breast cancer cell line, ZR75-1 (3). 4-OH TAM is a substrate for SULT1A1; consequently, SULT1A1 activity may be an important determinant in the efficacy of tamoxifen treatment, and modulation of SULT1A1 by genetic and/or environmental factors (i.e. genetic polymorphisms or induction by tamoxifen) could potentially have a profound influence on therapeutic outcome. Genetic polymorphisms in *SULT1A1* have been identified (4, 5), and one polymorphism in exon 7 of the *SULT1A1* gene results in an amino acid change from arginine to histidine at the conserved 213 position. This polymorphism has functional consequences for the translated protein in that the variant allele (His213, *SULT1A1*2*) has lower catalytic activity and decreased thermostability (4, 6). Investigations into the functional effect of *SULT1A1* polymorphisms on the phenotype of the enzyme in humans are largely carried out using platelets. Human platelets contain SULT1A1 enzyme, and SULT1A1-catalyzed enzymatic activity in platelets correlates closely with activities found in other tissues, displaying coordinate regulation (7-9). This correlation, and the easily accessible nature of platelets, has led to their widespread use in population studies. In our laboratory, we have demonstrated that platelet SULT1A1 activity correlates well with *SULT1A1* genotype, although the genotype only accounts for approximately 30% of the variation seen in phenotype (10). Gender was a significant determinant of phenotype ($p = 0.0001$), with females showing higher SULT1A1 activity than males. We have also examined *SULT1A1* genotype in DNA extracted from normal tissue in archived paraffin blocks from patients diagnosed with incident, primary, invasive breast cancer undergoing surgery and subsequent tamoxifen treatment. When the Kaplan-Meier function for survival by *SULT1A1* genotype was plotted, the high activity *SULT1A1*1* common allele was significantly associated with survival in the tamoxifen treatment group (11). This association was not seen in a group of patients who were receiving other treatments; therefore, *SULT1A1* genotype appears to affect long-term survival of patients treated with tamoxifen. These findings, along with information in the literature led to the following hypothesis. We hypothesized that the up-regulation of SULT1A1 mRNA expression by 4-OH TAM results in increased SULT1A1 protein and increased enzymatic activity, facilitating the elimination of environmental estrogenic compounds and the elimination of estrogens within breast tumors. Furthermore, the magnitude of this effect will be dependent on *SULT1A1* genotype. To test this hypothesis, we proposed the following objectives.

Objective one was designed to determine whether the reported *in vivo* induction of SULT1A by 4-OH TAM also resulted in an increase in expressed protein and enzymatic activity toward environmental estrogens in tamoxifen treated breast cancer patients. Objective two sought to determine the effect of 4-OH TAM on SULT1A1 activity in breast cancer cell lines. In objective three, genotyping for SULT1A1 polymorphisms and genotype-phenotype correlation studies were proposed, and in objective four, analysis of dietary data from the Block 98 questionnaire will be examined.

BODY

The following tasks were defined in the Statement of Work:

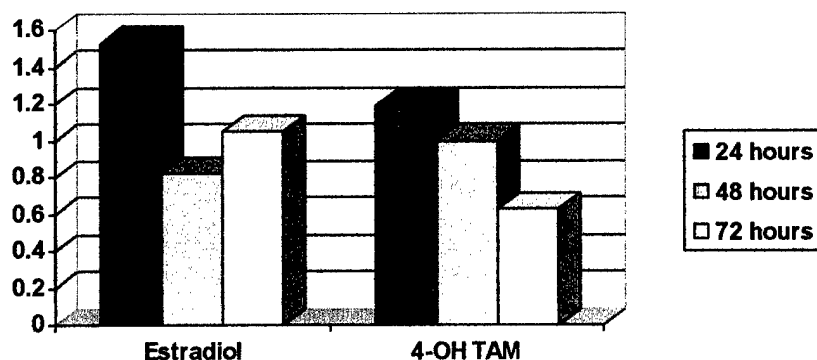
1. Determine if the induction of SULT1A1 by 4-OH-tamoxifen results in a global increase in the protein expression and activity of this enzyme toward environmental estrogenic compounds (Months 1 – 36).
2. Determine the effect of induction of SULT1A1 by 4-OH TAM in breast tumor cell lines on the activity of SULT1A1 toward estrogens. Examine the contribution of the estrogen or progesterone receptor status of the cell line to the induction (Months 1 – 30).
3. Determine SULT1A1 genotype of the study participants (see Task 1) and examine the genotype/phenotype correlation of tamoxifen-treated women (Months 30 – 33).
4. Collection of demographic and dietary questionnaire data for study participants, statistical analyses, and establishment of a bank of archived samples for future studies (Months 1 – 36).

Task 1 – In task one, it was proposed that over the three year period, 150 patients with primary, incident breast cancer who will be treated with tamoxifen will be recruited into the study. Recruitment for the study began on December 1, 2003. To date, 49 breast cancer patients have been recruited and have provided a blood sample and completed the Block 98 dietary questionnaire. 22 of these patients have also provided a repeat blood sample. The blood components have been fractionated and stored at liquid nitrogen temperatures as follows: five 3.5 ml aliquots of plasma, four 0.5 ml aliquots of serum, two aliquots of red blood cells, one blood clot, four aliquots of pelleted lymphocytes, four aliquots of pelleted platelets and four aliquots of pelleted neutrophils.

Since we will assess SULT1A1 enzymatic activity from platelets both before receiving tamoxifen and after tamoxifen levels have reached steady state in the circulation, the decision was made to store the platelets in several aliquots at liquid nitrogen temperatures. When the recruitment goal of 150 patients is attained and all samples are collected, cytosol will be prepared and enzymatic activity assays will be performed. By performing the assays on a complete sample set using the same reagents (sample buffer, PAPS, etc), this will allow for comparisons without the possible influence of variation in preparation of reagents. Studies in our lab have shown that SULT1A1 enzymatic activity in platelets is stable for more than five years when the platelets are stored in liquid nitrogen.

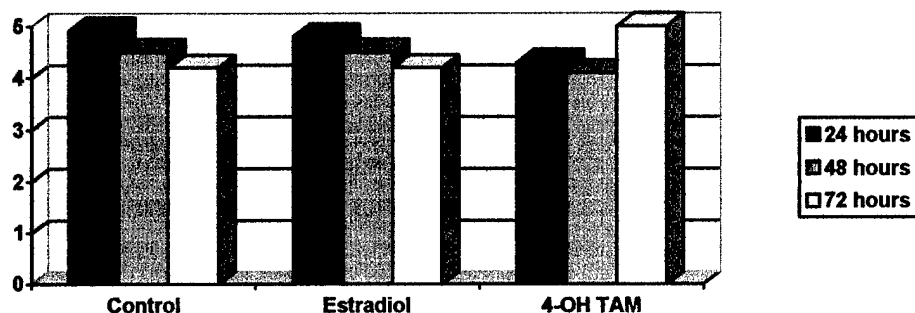
Task 2 – The breast cancer cell lines MCF-7 and ZR75-1 have been cultured to examine the effect of 4-OH TAM on the expression of SULT1A1. Cells were exposed to 10 nM estradiol and 5 μ M 4-OH TAM and harvested at 24, 48 and 72 hour time points. Total RNA was extracted and RT-PCR was performed using specific primers for SULT1A1 expression. Expression was quantified by densitometry and the relative intensity of the PCR products was normalized to the expression of β -actin in the same reaction. Figure 1 shows the expression of SULT1A1 relative to control incubations. When RNA was isolated from cells exposed to 10 nM estradiol, there was no evidence that expression of SULT1A1 increased over time with this exposure. Although there was no clear pattern, a slight decrease in expression over time was noted. When cells were exposed to 4-OH TAM, a similar pattern was observed, with a slight decrease in expression when the cells were exposed to 4-OH TAM. When the MCF-7 cell line was subjected to the same treatment, there was no evidence that either estradiol or 4-OH TAM increased SULT1A1 transcript (data not shown).

Figure 1. RT-PCR analysis of SULT1A1 expression in ZR75-1 cells.



It is known that expression of mRNA does not have a one to one correlation with the expression of protein. Regardless in changes in expression, if exposure to a substrate does not result in increased enzymatic activity of the protein, then the function of the protein is unaltered. Therefore, the same exposure experiments were performed, cells were harvested and cytosols were prepared for enzymatic activity assays. SULT1A1 activity was assessed as previously described (12). Figure 2 shows the sulfotransferase enzymatic activity of ZR75-1 cytosols toward 2-naphthol. Cells were exposed to 10 nM estradiol or 5 μ M 4-OH TAM for 24, 48 or 72 hours.

Figure 2. SULT Activity in ZR75-1 cells exposed to estradiol or 4-OH TAM



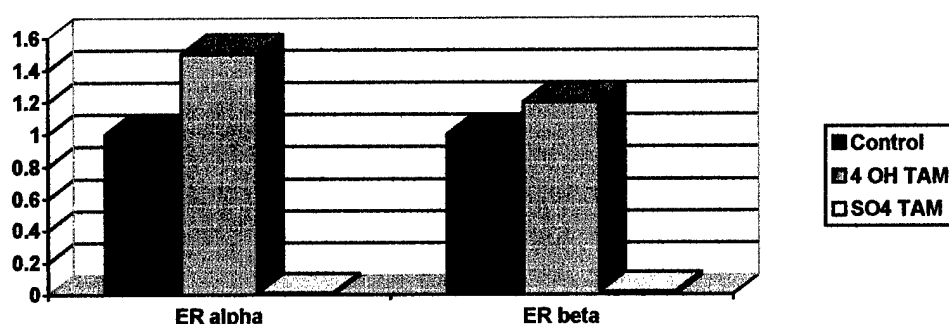
There was a slight decrease in sulfotransferase activity over 72 hours in cells receiving no treatment. When the cells were exposed to estradiol, there was no effect of this exposure on sulfotransferase activity. Treatment of cells with 4-OH TAM resulted in a slight increase in sulfotransferase activity toward 2-naphthol. When these experiments were repeated with MCF-7 cells, the results obtained were similar (data not shown). These data suggest that SULT1A1 expression and enzymatic activity are not affected by estradiol or tamoxifen exposure. Studies are continuing to examine the effect of these treatments on other breast cancer cell lines.

Task 3 – Lymphocytes have been isolated from the study participants for the genotype-phenotype studies proposed for Task 3. Once recruitment is complete, these studies will be performed.

Task 4 – Block 98 questionnaires have been completed for all study subjects recruited thus far. At the end of this year, these questionnaires will be submitted to Berkeley Nutrition Services for analysis.

Other studies – When overall survival was examined in breast cancer patients receiving tamoxifen, individuals who were homozygous for the *SULT1A1**2 low activity allele had significantly poorer overall survival than those who had one or two common alleles. The previous data suggests that exposure to tamoxifen does not increase expression of *SULT1A1*. Therefore, we explored other potential mechanisms of action. While sulfated estrogens are poor ligands for the estrogen receptor, it was possible that sulfation might beneficially alter the receptor binding properties of 4-OH TAM. To investigate this possibility, we synthesized 4-SO₄ TAM and performed receptor binding assays using a radioligand displacement assay and recombinant ER α and ER β purchased from Panvera Corporation. Figure 3 shows the results of these assays. When radioligand binding of estradiol is set as the control, as expected, 4-OH TAM had greater affinity for both ER α and ER β . When 4-SO₄ TAM is used, however, this compound showed no affinity for either receptor.

Figure 3. Receptor binding properties of 4- OH TAM and 4-SO₄ TAM



These data suggest that if sulfation of 4-OH TAM is beneficial, as suggested by the increased survival of patients with the high activity *SULT1A1* allele, the mechanism of action is not mediated by the estrogen receptor.

KEY RESEARCH ACCOMPLISHMENTS

Patient accrual for the study was projected to be approximately 50 patients per year. Recruitment of patients is on track with estimates (49 patients recruited thus far). Of the eligible patients who were approached to participate in the study, only one individual has refused. Isolation and storage of blood components for the study has presented no problems, and adequate amounts of material have been obtained for the proposed assays.

REPORTABLE OUTCOMES

No reportable outcomes have been generated from these studies thus far.

CONCLUSIONS

This study is proceeding according to plan, and the recruitment of study subjects will exceed study goals in the first year. The establishment of a bank of biological specimens has been accomplished, and these specimens will be crucial for the advancement of the Principal Investigator's research career. Once sample collection is complete and the assays are performed, the data generated will shed light on the role of SULT1A1 and response to tamoxifen for breast cancer therapy. These studies could contribute to the development of individualized therapy for breast cancer patients, and contribute to increased survival of this disease.

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